ELECTROPHORETIC ANALYSIS OF BLOOD OF *TILAPIA LEUCOSTICTA* TREWAVAS AND *TILAPIA ZILLII* (GERVAIS) FROM LAKE VICTORIA

By T. D. Iles and G. J. Howlett,
Fisheries Laboratory, Lowestoft

Introduction

Zone electrophoresis is one of the most useful tools now available for the separation of proteins in tissue fluids of organisms; it allows the examination of the variability of specific proteins among taxa and the comparison of proteins of one organism with another. In recent years there has been a marked increase in the application of electrophoretic techniques to taxonomic problems which has demonstrated that the comparison of proteins can add to more traditional criteria a sensitive means of characterizing taxonomic groups at all levels and of elucidating relationships between them.

Little has been done on African freshwater fishes, and amongst these no group offers material of greater interest and value than do the cichlids, which provide interesting problems in evolution and considerable difficulty in identification by traditional criteria. This communication represents a preliminary account of work done on two species of the genus *Tilapia* and concerns the results of the analysis of material sent by EAFFRO to the Lowestoft Fisheries Laboratory in August 1966.

Both the species involved are recent introductions into Lake Victoria. *T. zillii* has proved a successful colonizer and has formed populations large enough to make a small but significant contribution to commercial catches; *T. leucosticta* has achieved its more limited success by its ability to withstand the relatively adverse conditions found in the marginal lagoons and swamps of the lake (Welcomme 1964a).

Whole blood was collected from the heart or sinus by Vacutainer tubes [illegible] Lowestoft Laboratory within 24 hours. The samples were centrifuged at 2,500 r.p.m. at 0°C for 20 minutes and the supernatant plasma removed. The red cells were washed twice in 1.14 percent saline before being lysed

in about four times their volume of phosphate buffer. After final centrifugation the clear haemolysate was separated from the cell stroma and was ready for electrophoresis.

Thirteen individuals of each species were sampled, together with one considered to represent, possibly, a hybrid between *T. zillii* and *T. melanopleura* (Welcomme pers. comm.).

Horizontal starch-gel electrophoresis was carried out on both haemoglobin and plasma proteins, the method being essentially that used by Smithies (1955) and as described by Jamieson (1965). The starch concentration was 12 percent (Connaught hydrolyzed starch), and a continuous buffer system of Tris-EDTA-Boric acid at pH 9.1 was used. A charge of 400 volts was applied for 4 or 5 hours at a temperature of about 2°C inside a domestic refrigerator.

Haemoglobins were analysed also by agar-gel electrophoresis, using Ion-Agar No. 2 agar and a phosphate buffer at pH 7.3 as described by Sick (1965). Standard 75 mm x 25 mm slides supported the gel and a current of 5ma per slide was applied for 40 minutes, again at about 2°C.

Both types of gel were fixed in a methanol/acetic acid/water medium (5:1:5 by volume). Agar gels were dried in a current of warm air and all gels were stained in the general protein stain Amido-black 10b—at a concentration of 7 g/litre of fixing fluid.

Results

Plasma proteins

The starch-gel electrophoretic patterns of plasma proteins of both *Tilapia zillii* and *T. leucosticta* exhibit a relatively high degree of individual variability. Plate H1 illustrates patterns for individual fish, together with that for human plasma as a reference.

There is an overall similarity in band pattern between the species, but despite this—and despite the individual variation within species—it is possible to see characteristic patterns for each species (or rather for the populations of the species represented in the samples).

The staining technique is a general one for "total" proteins, so that the resulting band pattern represents a complex mixture of plasma proteins belonging to a number of protein systems which have not yet been identified separately in cichlid material. The use of prominent or characteristic bands in the electropherograms to delimit zones is, therefore, a matter of convenience only.

Migration of proteins is towards the anode and the terms "fast" and "slow" indicate the relative migration rates in this direction. The four bands marked A, B, C and D in Plate H1 form the basis of the characteristic *T. zillii* pattern, and of these bands A, C and D are usually prominent. Band B is not usually conspicuous but it was nearly always present in a characteristic position. For individuals of *T. leucosticta* a band with the same migration rate as B was usually more prominent; a prominent band corresponding to C in *T. zillii* was not found, and a prominent slow band equivalent to A was slightly faster.

Other differences between the two species are found in proteins faster than D, which may well represent or include the plasma albumens. These bands are rather diffuse and are not well shown in the Plate, but repeated comparisons have indicated that the fastest protein fraction for *T. leucosticta* lies between the position of two separate bands in *T. zillii* material.

Within each species individual variation involves differences in the relative intensities of bands at specific migration distances, the appearance of extra bands or the apparent absence of "characteristic" bands. Variation of these kinds appears at each of the characteristic positions for both species.

Haemoglobins

Electrophoresis in agar-gel at pH 7.3 causes the haemoglobins of *T. zillii* and *T. leucosticta* to migrate towards the cathode and to separate into a number of components – as many as nine being identified. The existence of multiple haemoglobins is quite prevalent in both vertebrates and invertebrates, but the number now revealed for these two species is much greater than that demonstrated for *T. mossambica* by Chandresekhar (1959); he found one major and one minor component. Examination of the haemoglobin for a number of species of *Tilapia* under agar-gel electrophoresis, including *T. mossambica*, confirms that the larger number of haemoglobins found in *T. zillii* and *T. leucosticta* is characteristic for *Tilapia* (unpublished data).

In both species there is individual variation, in that components of specific migration rates may vary greatly in intensity to give recognizably distinct overall patterns. Although up to nine separate bands are involved, with a potentially larger number of intergrading patterns in both species, the patterns tend to fall into a small number of more or less discontinuous groups which probably represent the phenotypic expression of a genetically based polymorphism.

There are differences between the two species which involve the migration rates of all of the haemoglobin components. Parallel runs of

haemoglobins of *T. zillii* and *T. leucosticta* indicate that some at least, and probably all, of the individual components are displaced relative to each other, as is shown in Plate H2a.

The electrophoretic behaviour of fish haemoglobins tends to be affected greatly by changes in the pH of the buffer system. At pH 9.1 – that of the buffer used in starch-gel electrophoresis – nearly all of the haemoglobin bands migrate to the anode, so that bands nearest the point of application for agar-gel electropherograms migrate as the fastest components under starch-gel electrophoresis with the Tris-EDTA-Boric acid buffer.

Starch-gel electrophoresis at pH 9.1 did not give as fine a resolution of individual haemoglobins as did agar-gel, but the results do facilitate comparisons between the two species.

In Plate H2b the haemoglobins of individuals from both species are compared. Two patterns can be distinguished which are labelled A and B. Type A consists of single relatively narrow intense bands; in Type B the single bands of A are represented by broader diffuse bands which in some cases can be resolved into more than one component.

For both species individuals can be referred to Type A or B, but Plate H2b shows that anodic migration rates for the haemoglobins of *T. zillii* are greater than those for corresponding bands of *T. leucosticta*. The slowest band for *T. zillii* remains at the point of application, whereas that of *T. leucosticta* has a small cathodic migration rate. The fastest anodic band of *T. zillii* always migrates further than that of *T. leucosticta*. These differences are found for both A and B patterns.

The importance of this observation lies in the fact that the two species being compared belong to different subgenera, *T. zillii* representing the substrate-spawning subgenus *Tilapia* and *T. leucosticta* the mouth-brooding subgenus *Sarotherodon*. This raises the possibility that the electrophoretic differences in the haemoglobins may serve to distinguish the subgenera themselves, a possibility that tends to be confirmed by studies of haemoglobins of other species of the genus *Tilapia* (unpublished data).

Discussion

Although the results dealt with in this communication involve only two species and a relatively small number of individuals, they do indicate the kind of information that might result from wider studies. The populations that were sampled exhibit a great deal of individual variation, demonstrated more obviously by the plasma proteins, and of a type that is well suited for the investigation of problems at the intra-specific level (Dessauer 1966). Some of the variability could be the result of developmental changes which could be interpreted in life-history terms, but this is unlikely to be

the explanation in the case of *T. leucosticta,* the samples of which were taken from fish over a very small length range – all but one falling between 13 and 16 cm total length. The length range for *T. zillii* was larger, from 11.0 cm to 24.8 cm, but there was no indication that any correlation existed between electropherogram pattern and fish length for either plasma proteins or haemoglobin. Nor is it likely that a significant proportion of the variability represents the effects of differing environmental factors, because the samples were taken from a relatively small area of the lake – presumably from single populations.

Tsuyuki and Roberts (1965) concluded that serum proteins of northern freshwater fishes show more individual variation than do those of northern marine fishes, so that the variability in these two species of *Tilapia* may represent a characteristic feature of freshwater forms as a whole, However, it seems more likely and more interesting to postulate that part at least of the diversity and the underlying genetic heterogeneity that it implies is associated with the fact that these two species are recent colonizers. Since about 1951, at different places in Lake Victoria and on a number of separate occasions, each species has been introduced from populations taken originally from other East African lakes, but which have often been held for a time in small dams or ponds (Welcomme 1964b). The relatively low selection pressures they have presumably experienced in their new environment may well have allowed or favoured a relatively high degree of variability.

A comparison of different populations of species of *Tilapia* which have been introduced into Lake Victoria, of which there are several (Welcomme 1964b), with those of endemic species would test such a hypothesis. The electrophoretic technique certainly represents an almost unique opportunity of studying colonizing species to a degree beyond that at which more traditional morphological criteria can reasonably be expected to give results.

Although species specificity is indicated in both plasma proteins and haemoglobins it is not possible, on the basis of this material alone, to determine to what extent zone electrophoretic methods can be expected to contribute to the solution of taxonomic problems at the specific or higher levels. Results from more extensive studies on a number of *Tilapia* species from adjacent regions of Africa indicates that much useful diagnostic information should be forthcoming even when relatively few types of protein systems are analysed. In particular the more "conservative" proteins such as the muscle myogens (Tsuyuki, Roberts and Vanstone 1965) promise to be of considerable value, both for the genus *Tilapia* itself and for members of the cichlid flocks found in Lake Victoria and in other African Lakes.

REFERENCES

DESSAUER, H. C. (1966) – Taxonomic significance of electrophoretic patterns of animal sera. *Serological Museum, Bull. 34:* pp 4–8.

CHANDRESEKHAR, N. (1959) – Multiple haemoglobins in fish. *Nature, 184:* 1652–1653.

JAMIESON, A. (1965) – The genetics of transferrins in cattle. *Heredity 20* (3) : 419–441.

SICK, K. (1965) – Haemoglobin polymorphism of cod in the North Sea and the North Atlantic Ocean. *Hereditas, 54 :* 49–73.

SMITHIES, O. (1955) – Zone electrophoresis in starch-gels *Biochem. J., 61 :* 629.

TSUYUKI, H. & ROBERTS, E. (1965) – Zone electrophoretic comparison of muscle myogens and blood proteins of artificial hybrids of Salmonidae with their parental species. *J. Fish. Res. Bd. Canada, 22* (3) : 767–773.

TSUYUKI H. & ROBERTS, E. & VANSTONE, W.E. (1965) – Comparative zone electropherograms of muscle myogens and blood haemoglobins of marine and freshwater vertebrates and their application to biochemical systematics. *J. Fish. Res. Bd. Canada, 22* (1) : 203–213.

WELCOMME, R.L. (1964a) – The habits and habitat preferences of the young of the Lake Victoria Tilapia (Pisces-Cichlidae). *Rev. Zool. Bot. afr., 70* (1–2) : 1–28.

WELCOMME, R.L. (1964b) – Notes on the present distribution and habits of the non-endemic species of *Tilapia* which have been introduced into Lake Victoria. *Ann. Rep. EAFFRO.* 1962/63, App.D. : 36–39.

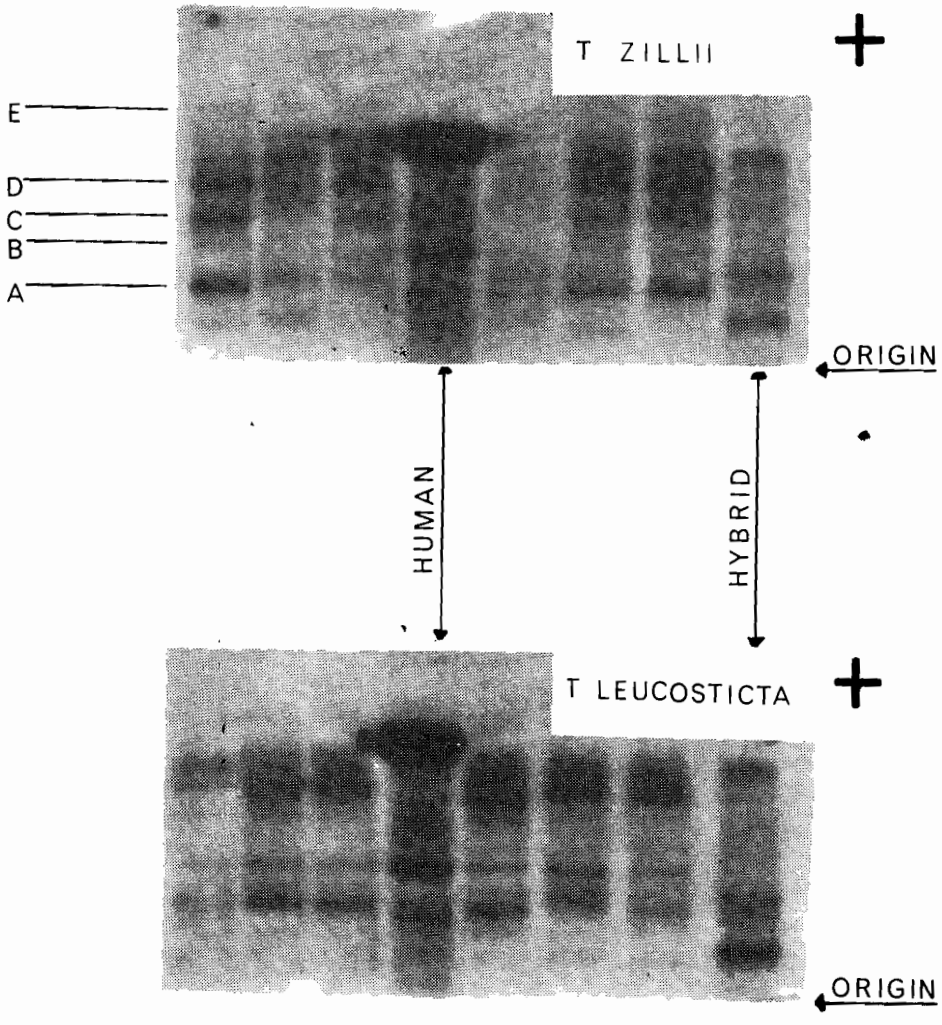

PLATE H 1. Starch-gel electrophoresis of plasma proteins of individuals of T. zillii and T. leucosticta, together with those of an individual thought to be a hybrid between T. zillii and T. melanopleura. Human plasma is included as a reference.

TRIS – EDTA – boric acid gel pH 9.1, stained with Amido Black 10b. The positions of "characteristic" T. zillii bands A B C and D are indicated. The fastest systems (possibly albumens) are in the region marked E. Note the differences in intensities of bands at position B between the two species; at position A, comparison with a prominent band in human plasma illustrates another specific difference. Note also individual differences particularly at positions A and C.

PLATE H 2a. Agar-gel electrophoresis of haemoglobins of T. zillii and T. leucosticta. Phosphate gel pH 7.3 (Sick) stained with Amido Black 10b. Migration of all haemoglobins is cathodic. Comparison of migration rates of haemoglobin components of T. zillii and T. leucosticta is made in the middle of the gel where they are not subject to distortion.

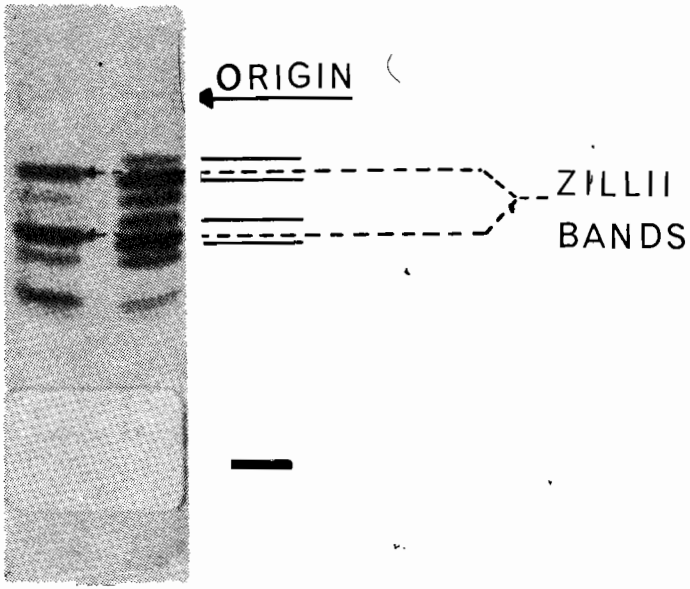

PLATE H 2b. Starch-gel electrophoresis of haemoglobins of T. zilli and T. leucosticta. TRIS – EDTA – boric acid gel pH 9.1, stained with Amido Black 10b. Most haemoglobin components migrate to the anode. Two patterns are recognized, labelled A and B. A difference in migration rate is apparent whether A's or B's are compared between species.